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DIFFERENTIAL NUMBERS OF FOCI OF LYMPHOCYTES WITHIN THE BRAINS OF LEWIS RATS EXPOSED TO WEAK COMPLEX NOCTURNAL MAGNETIC FIELDS DURING DEVELOPMENT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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To discern if specific structures of the rat brain contained more foci of lymphocytes following induction of experimental allergic encephalomyelitis and exposures to weak, amplitude-modulated magnetic fields for 6 min once per hour during the scotophase, the residuals between the observed and predicted values for the numbers of foci for 320 structures were obtained. Compared to the brains of sham-field exposed rats, the brains of rats exposed to 7-Hz 50 nT (0.5 mG) amplitude-modulated fields showed more foci within hippocampal structures and the dorsal central grey of the midbrain while those exposed to 7-Hz 500 nT (5 mG) fields showed greater densities within the hypothalamus and optic chiasm. The brains of rats exposed to either the 50 nT or 500 nT amplitude-modulated 40-Hz fields displayed greater densities of foci within the midbrain structures related to rapid eye movement. Most of the enhancements of infiltrations within the magnetic field-exposed rats occurred in structures within periventricular or periaqueductal regions and were both frequency- and intensity-dependent. The specificity and complexity of the configurations of the residuals of the numbers of infiltrated foci following exposures

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to the different fields suggest that the brain itself may be a "sensory organ" for the detection of these stimuli.

Keywords Amplitude-modulated magnetic fields, central grey, experimental allergic encephalomyelitis, optic chiasm, rat brain 7 Hz 40 Hz, rapid eye movement (REM)

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In previous studies Cook and Persinger (1999) had hypothesized quantitative measures of the perivascular infiltration of lymphocytes into the brain could be employed as sensitive markers for the selective vulnerability of specific nuclei to an experimental treatment. Rats sensitized with spinal cord emulsion and injected with dosages of lithium and pilocarpine sufficient to evoke seizures exhibited multifocal neuronal loss and pervasive infiltration of lymphocytes within those regions of the brain. The rats that had received subclinical dosages of lithium and pilocarpine (no seizures) exhibited no discernable neuronal loss but displayed very clear infiltrations of lymphocytes within limbic tracts such as the fornix.

Persinger, Cook, and Koren (2000) reported that whole-body exposures to an amplitude-modulated (1 nT to 50 nT) 7-Hz square-wave magnetic field generated through Helmholtz coils for 6 min per hour between midnight and 08:00 hr (late scotophase) resulted in an attenuation of the symptoms of Experimental Allergic Encephalomyelitis (EAE). The exposures began on the day of inoculation and continued until the rats were killed about five days after the onset of the clinical symptoms. The effect size of the treatment accommodated more than 50% of the variance in the clinical scores.

Subsequent studies (Cook & Persinger, 2000) indicated that whole body nocturnal exposures to the same temporal structure of 7-Hz magnetic fields with intensities of 500 nT (5 mG) or to 40-Hz magnetic fields within either the 50 nT or 500 nT range with this temporal structure resulted in no clinical benefits. Rats that had been exposed to the 7-Hz, 50 nT amplitude-modulated magnetic field, in addition to the suppressed clinical symptoms, also showed significantly fewer foci of lymphocytes within the brain compared to rats that had been exposed to sham fields or to the other intensities. In that study the means of the numbers of foci within traditional and larger divisions of the brain, such as the thalamus or midbrain, were assessed.

In the present study an orthogonal approach was employed. All of the structures ($n = 320$) differentiated by Paxinos and Watson (1986) within the rat brain were employed as the cases for analyses and the various treatments (7-Hz or 40-Hz fields presented as either 50 nT or 500 nT intensities) were designated as variables. Consequently, by correlating the numbers of foci in each structure

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between each treatment and the reference group regression analyses could be applied to discern those structures whose residuals were significantly above or below the predicted values from the reference (control) group. This technique would allow a more precise isolation of where within the volume of the brain the different treatments specifically affected the penetration or accumulation of the sensitized lymphocytes.

METHODS AND MATERIALS

Subjects

Fifty-six female Lewis rats (aged 16–20 weeks old), obtained from Charles River, Inc (Montreal, Quebec), were employed as subjects in eight separate blocks of experiments.

Exposure

All rats were injected in both hindfoot pads with fresh female Lewis rat spinal cord emulsified in Freund's complete adjuvant (40 mg/kg; 0.1 cc injection/footpad). Immediately following inoculation, the rats were weighed and placed two per cage. Each of two plastic cages (32 cm by 36 cm by 45 cm) was surrounded along its major axis by a coil (30 ohms) of wire (AWG 20). The two coils were connected to a custom built output for a Model EU 81A Heath-Schlumberger function generator. Two other identical cages were maintained immediately 30 cm below the two coils and housed the reference groups Persinger et al. (2000)

When the field was activated by the Commodore computer, the intensity increased in sequential steps 7 times during the first 3 min and 20 s and 7 times during the last 1 min and 42 min. The duration of each plateau was 14 s during the first cluster and 7 s during the last cluster. The time required to change to each increment of intensity was 2 microsecond. The pattern has been published elsewhere (Cook & Persinger, 2000). The total exposure time was 5 min and 50 s every hour from 00:00 hr to 07:06 hr. The temperature within the room which housed the apparatus and rats was automatically controlled within one degree of 20°C. The light:dark cycle was 12:12 with the onset time of 08:00 hr. Blocks consisted of four rats exposed within the two coils and four rats housed within the reference area below the coils.

For each of four nonconsecutive blocks either a square-wave 7-Hz field or square-wave 40-Hz field was generated through the coils. For two of each of these blocks the average peak intensity within the coils during the 6 min pattern

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was either about 50 nT (+/−10%) or 500 nT (+/−10%). The occurrence of the presentation of the fields was verified by the computer log that recorded both the time and status of the coils. The presence and intensities of the fields were calibrated before the beginning of each block and measured (verified) every second night with a Metex M-3800 digital multimeter and sensory [Electric Field Measurement, W. Stockbridge, MA 01266]. During the application of the 500 nT 7-Hz and 40-Hz within the solenoids, extended fields within the two reference cages averaged between 25 nT and 30 nT.

The occurrence of these fields allowed a test to eliminate the possibility that any magnetic field effects might be artifacts of the presence of the coils around the exposure cages. If the effects on the overt symptoms of EAE for rats in the reference cages (about 30 nT) when the extended fields generated by the 500 nT fields were similar to those exposed within the coils during the generation of the 50 nT fields, then confounding variables associated with caging or position within the rack would be minimal. When the 50 nT fields were generated within the coils, the intensities within the lower (reference) cages never fluctuated and were less than 10 nT (limits of the sensor). The potential penetration of intermittent magnetic fields from the adjacent room through the walls into the experimental cages was eliminated by prohibiting the use of electronic equipment within these areas. The exposure time for each block was 15 nights.

Histology

The rat brains were removed 15 days after the injection of the spinal cord into the footpads and 15 days after the initiation of the treatments. The brains were fixed in ethanol-formalin-acetic acid and processed. Ten micrometer sections were obtained between the caudal medulla and the rostral telencephalon (anterior commissure). Every 20th section was stained with toluidine blue O. The numbers of foci of lymphocytes, almost exclusively around blood vessels, for each of the 320 structures discerned by Paxinos and Watson (1986) between the caudal medulla and anterior commissure were counted at 100× and 400× magnification.

A focus was defined as a discriminable (usually more than 10) cluster of darkly stained, lymphocytic-looking cells around a blood vessel or a likely blood vessel. Identification of foci was clear because such clusters of these cells are rarely found in normal Lewis brains. The inter-rater correlation for approximately 12 different sites of foci on five successive slides was 0.96.

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Statistical Analyses

In this study the structures (rather than animals) were the cases and the rats were the variables. The numbers of foci per structure were loaded for computer analysis for each of the 320 brain structures for each of the rats. The means of the numbers of foci were calculated separately for the right side of the brain and the left side of the brain. The numbers of animals in each treatment from which the means were calculated were: sham field (reference) group: $n = 15$, 7-Hz 50 nT group: $n = 15$, 7-Hz 500 nT group: $n = 8$, 40-Hz, 50 nT group: $n = 13$, 40-Hz, 500 nT group: $n = 5$. Consequently there were 5 means for the numbers of foci within each structure for each of the two sides of the brain.

Regression analyses between the numbers of foci in the left and the right side of the brain for each of the four magnetic field treatments and the values for the left and right side of the brain, respectively, for the sham field group were completed by SPSS software on a VAX 4000 computer. As a result, there were eight regression analyses. The standardized residuals for the discrepancies in the numbers of foci between the predicted values based upon the values for the sham field group and the actual values for each of the two sides of the brain for each of the four treatments were employed as the data. Only discrepancies of more than three standard deviations were accepted as significant from a "clinical" perspective.

The differences between numbers of foci per structure and the density of foci per structure do not affect the meaning or interpretation of the correlations or the residuals between the sham-field exposed brains and those that had been exposed to the various experimental magnetic fields. However the density of foci within various structures, upon which the experimental treatments would be superimposed, may be important for discerning potential mechanisms. Consequently, the density of the foci per mm^2 was calculated by dividing the numbers of foci per structure by its cross-sectional area.

RESULTS

The means and standard deviations for the numbers of foci per mm^2 for the 100 Paxinos and Watson structures with the highest density of foci of lymphocytes are shown in Table 1. Many of the remaining 220 structures that were assessed either did not display foci or the values for the foci were due primarily to one or two rats. The most concentrated numbers of foci occurred within

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Table 1. Means and standard deviations for the numbers of foci per mm^2 for the 100 structures (Paxinos–Watson abbreviation) with the greatest density in brains of rats in which experimental allergic encephalomyelitis had been induced and were exposed to sham-field conditions. The structures for which data for areas were available are ordered from greatest to least density

Abrev.	*M*	*SD*	Full name
Pe	97.0	4.7	Periventricular hypothalamic nucleus
Mhb	74.8	26.2	Medial habenular nucleus
PVA	53.5	9.7	Paraventricular thalamic nucleus (anterior)
f	44.0	10.1	Fornix
ox	41.4	.6	Optic chiasm
SCh	35.5	10.7	Suprachiasmatic nucleus
sm	32.9	6.5	Stria medullaris of the thalamus
PT	32.5	10.6	Paratenial thalamic nucleus
PaMP	26.5	4.5	Paraventricular hypo. nuc. (medial parvocellular)
ml	24.5	.7	Medial lemniscus
Arc	22.3	12.7	Arcuate hypothalamic nucleus
MnPO	21.5	1.0	Median preoptic nucleus
Xi	19.4	.9	Xiphoid thalamic nucleus
SFi	17.5	10.6	Septofimbrial nucleus
PVP	14.6	2.9	Paraventricular thalamic nucleus (posterior part)
fi	13.1	1.0	Fimbria
LSV	13.1	9.7	Lateral septal nucleus (ventral part)
MnR	12.9	.6	Median raphe nucleus
hbc	12.9	.6	Habenular commissure
opt	12.8	2.5	Optic tract
BSTMPM	12.5	17.7	Bed nuc. Stria terminalis (medial, posteromedial)
BSTS	9.5	4.1	Bed nuc. Stria terminalis (supracapsular division)
AD	8.6	.4	Anterodorsal thalamic nucleus
VTg	7.9	6.5	Ventral tegmental nucleus (von Gudden)
LHbM	7.8	11.0	Lateral habenular nucleus, medial part
AHP	7.8	11.0	Anterior hypothalamic area (posterior part)
Re	7.7	1.5	Reuniens thalamic nucleus
me5	6.7	.8	Mesencephalic trigeminal tract
MDC	6.7	9.4	Mediodorsal thalamic nucleus (central part)
VRe	6.7	9.4	Ventral reuniens nucleus of the thalamus
LSI	6.7	.0	Lateral septal nucleus (intermediate)
AVDM	6.6	4.9	Anteroventral thalamic nucleus (dorsomedial)
IAM	6.5	.3	Interanteromedial thalamic nucleus
IPL	6.4	2.0	Interpeduncular nucleus (lateral subnucleus)
AM	6.4	3.4	Anteromedial thalamic nucleus
MPO	6.4	1.2	Medial preoptic nucleus
BSTMA	6.3	4.3	Bed nuc. Stria terminalis (medial div., anterior)
HDB	6.3	4.3	Nucleus of the horizontal limb of diagonal band
AVVL	6.2	8.8	Anteroventral thalamic nucleus (ventrolateral)

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Table 1. Means and standard deviations for the numbers of foci per mm^2 for the 100 structures (Paxinos–Watson abbreviation) with the greatest density in brains of rats in which experimental allergic encephalomyelitis had been induced and were exposed to sham-field conditions. The structures for which data for areas were available are ordered from greatest to least density (*Continued*)

Abrev.	*M*	*SD*	Full name
alv−m	6.0	5.6	Alveus of hippocampus
rs	5.6	3.4	Rubrospinal tract
dhc	5.4	.2	Dorsal hippocampal commissure
LPO	5.4	.2	Lateral preoptic area
RtTg	5.0	.2	Reticulotegmental nucleus of the pons
CST	5.0	7.1	Nucleus of the commissural stria terminalis
PC	4.9	2.5	Paracentral thalamic nucleus
Rt	4.5	3.1	Reticular thalamic nucleus
alv−l	4.4	.4	Alveus
ZI	4.2	5.9	Zona incerta
AMPO	4.2	5.9	Anterior medial preoptic nucleus
DG	3.9	.2	Dentate gyrus
DM	3.9	5.5	Dorsal medial hypothalamic nucleus
FSTr	3.7	5.3	Fundus striati
CG	3.6	.0	Central (periaqueductal) grey
tfp	3.5	.2	Transverse fibers of the pons
csc	3.4	.6	Commissure of the superior colliculus
PrS	3.3	4.7	Presubiculum
SO	3.3	4.7	Supraoptic nucleus
cp	3.2	1.1	Cerebral peduncle (basal part)
MZMG	3.2	.1	Medial zone of the medial geniculate
PV	3.2	.1	Paraventricular thalamic nucleus
MD	3.2	2.1	Mediodorsal thalamic nucleus
PaV	3.2	.8	Paraventricular hypothalamic nuc. (ventral part)
BSTV	3.2	1.4	Bed nuc. Stria terminalis (ventral part)
VDB	3.2	.1	Nucleus of the ventral limb of the diagonal band
tz	3.0	2.0	Trapezoid body
DA	3.0	.9	Dorsal hypothalamic area
PaAP	2.8	.1	Paraventricular hypoth. Nuc. (anter. Parvocell.)
SHy	2.8	.8	Septohypothalamic nucleus
ac	2.6	1.1	Anterior commissure
CGD	2.4	.1	Central grey (dorsal part)
mlf	2.3	3.3	Medial longitudinal fasiculus
LSD	2.3	.8	Lateral septal nucleus (dorsal part)
ts	2.2	1.6	Tectospinal tract
RPC	2.1	2.9	Red nucleus (parvocellular part)
VMH	2.1	1.3	Ventromedial hypothalamus
PaPo	2.1	2.9	Paraventricular hypothalamus (post. Part)

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Table 1. Means and standard deviations for the numbers of foci per mm^2 for the 100 structures (Paxinos–Watson abbreviation) with the greatest density in brains of rats in which experimental allergic encephalomyelitis had been induced and were exposed to sham-field conditions. The structures for which data for areas were available are ordered from greatest to least density (Continued)

Abrev.	*M*	*SD*	Full name
IPDL	1.9	.8	Interpeduncular nucleus (dorsolateral)
IPA	1.8	.1	Interpeduncular nucleus (apical subnuc.)
BSTMPI	1.8	2.5	Bed nucleus of stria terminalis, medial division, posteriorintermediate part
bic	1.7	.8	Brachium of inferior colliculus
BSTMPL	1.7	2.4	Bed nucleus of stria terminalis, medial division, posteromedial part
cc	1.6	.1	Corpus callosum
MGM	1.6	2.2	Medial geniculate nucleus, medial part
CM	1.6	.1	Central medial thalamic nucleus
PMV	1.6	.8	Premammillary nucleus, ventral part
SHi	1.6	2.2	Septohippocampal nucleus
df	1.4	.1	Dorsal fornix
ll	1.3	.2	Lateral lemniscus
fmj	1.2	.2	Forceps major of the corpus callosum
PeF	1.2	1.8	Perifornial nucleus
TC	1.2	.1	Tuber cinereum area
PL	1.1	1.5	Paralemniscal nucleus
PF	1.1	1.6	Parafasciular nucleus
LDDM	1.1	1.6	Laterodorsal thalamic nucleus, dorsomedial part
MDM	1.1	1.6	Mediodorsal thalamic nucleus, medial part
VM	1.1	1.6	Ventromedial thalamic nuclues
MPA	1.1	.0	Medial preoptic nucleus
Tu	1.1	1.6	Olfactory tubercule
Sta	1.1	.0	Strial part of the preoptic area
s5	1.0	.2	Sensory root of the trigeminal nerve
sox	1.0	.7	Supraoptic decussation
st	1.0	.7	Stria terminalis
BSTLJ	1.0	1.5	Bed nucleus of stria terminalis, lateral division, juxtacapsular part

diencephalic structures that included periventricular, paraventricular, arcuate, and suprachiasmatic nuclei of the hypothalamus, the fornix and optic chiasm, and the medial habenulae, paraventricular nucleus, paratenial nucleus, and stria medullaris of the thalamus.

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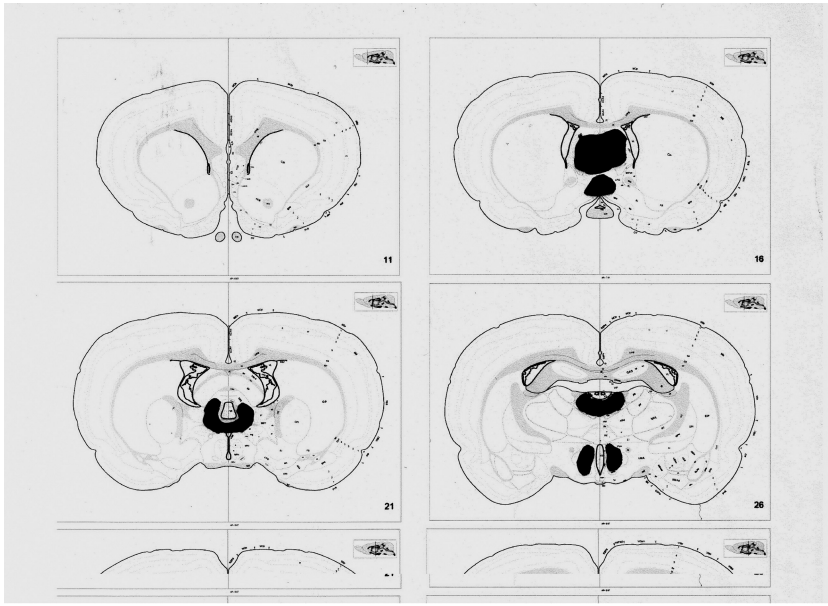

Figure 1. Spatial patterns within coronal sections of the brain where the observed numbers of foci of mononuclear cells (indicated by darkened areas) were more than three standardized residuals higher than the predicted values for all treatments.

The correlations between the numbers of foci in the brains of the rats that had been exposed to one of the four magnetic field treatments and the numbers of foci in the brains of the control rats ranged between .61 and .87 (all $p < .001$). The correlation coefficients between numbers of foci in control brains and those exposed to the 50 nT 40-Hz field or to either intensity of the 7-Hz fields ranged between .80 and .87. Z-transformations for the correlation coefficients indicated that the strength of the correlations ($rs = 0.61, 0.68$) between the group exposed to the 500 nT, 40-Hz fields and the control group were significantly weaker ($z = 6.54, p < .001$) than the strength of the correlations (average about 0.85) for the other groups. Inspection of the scattergrams indicated more or less linear relationships. The general distributions of structures, where the observed values for the numbers of foci were more than three standardized residuals higher than the predicted values, are shown for all conditions in Figure 1.

Table 2 shows the observed values and predicted values (based upon means from rats exposed to the sham-field condition) for the numbers of foci within the left and right sides of the brain for rats that had been exposed to the 500 nT

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Table 2. Structures whose discrepancies exceeded +/− 3 standard deviations between the observed numbers and predicted numbers of foci of infiltrations of lymphocytes for rats exposed nocturnally to amplitude-modulated 500 nT, 40 Hz magnetic fields relative to controls

Structure	Observed	Predicted	z-resid
Right Side (r = 0.68, S.E. = 0.32)			
Paranigral nuc.	2.74	1.74	3.14
Central periaqueductal grey	1.87	.81	3.24
Central tegmental tract	1.43	.17	3.86
Paratenial thalamic nuc.	1.83	.40	4.37
Left Side (r = 0.61, S.E. = 0.39)			
Lateral dorsotegmental nuc.	1.39	.21	3.04
Pontine reticular nuclei	1.83	.33	3.85
Subceruleus nucleus (dorsal)	1.52	.25	3.27
Deep mesencephalic nucleus	1.43	.21	3.16
Central periaqueductal grey	2.26	.84	3.65
Inferior colliculus (ex.ctx)	1.70	.45	3.22
Medial longitudinal fasic	2.39	.45	5.01
Superior cerebellar peduncle	1.39	.21	3.04
Paratenial thalamic nuc.	1.70	.37	3.42

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40-Hz amplitude-modulated fields following inoculation. Most of the structures that exhibited the greater numbers of foci relative to the sham-field exposed rats were within the more central core of the midbrain. The structure that showed the greatest numbers of foci within both sides of the brain compared to the control groups was the central periaqueductal grey.

Table 3 contains the observed values and predicted values for the numbers of foci within the left and right sides of the brain for rats that had been exposed to only 50 nT, 40-Hz amplitude-modulated magnetic fields following inoculation. The paratenial thalamic nucleus and the alveus, a component of the white matter of the hippocampal formation, contained more foci than expected on the basis of their concentrations within the sham-field (control) brains. The structure that displayed the greatest numbers of foci within both sides of the brain compared to the control group was the alveus.

The observed values, expected values, and standardized residuals in Table 4 show the effects of exposure to the 500 nT, 7-Hz amplitude-modulated magnetic fields. The structures containing disproportionately more foci than those in the sham-field-exposed rats were primarily within the hypothalamic region. The triangular nucleus of the septum as well as the optic chiasm displayed more foci in both sides of the brain than would be expected from sham-field exposures.

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Table 3. Structures whose discrepancies exceeded +/− 3 standard deviations between the observed numbers and predicted numbers of foci of infiltrations of lymphocytes for rats exposed nocturnally to amplitude-modulated 50 nT, 40 Hz magnetic fields relative to controls

Structure	Observed	Predicted	z-resid
Right side (r = 0.87, S.E. = 0.18)			
Alveus	.80	.24	3.05
Medial habenulae nucleus	1.86	1.22	3.44
Paratenial thalamic nuc.	1.00	.28	3.91
Anterior medial preoptic area	.66	.04	3.39
Septal fimbrial nucleus	.77	.16	3.34
Lateral lemniscus	.03	.69	−3.62
Paraventricular nuc. Thal. (ant.)	.54	1.14	−3.28
Left side (r = .83, S.E. = 0.21)			
Paranigral nucleus	2.51	1.77	3.61
Pontine reticular nuc. (oral)	1.23	.15	5.21
Alveus	1.06	.27	3.78
Paratenial thalamic nuc.	1.00	.19	3.90
Suprachiasmatic nuc.	1.20	.44	3.67
Stria medullaris of thalamus	.29	1.06	−3.78
Paraventricular nuc. Thal.(ant.)	.46	1.23	−3.76
Medial preoptic area	.09	.82	−3.56

The structure that exhibited the largest numbers of foci on both sides of the brain compared to the control groups was the optic chiasm.

Table 5 contains the observed values, expected values, and residuals for the numbers of foci in the brains of rats that had been exposed to the 50 nT, 7-Hz amplitude-modulated magnetic fields. There were more foci than expected within the optic chiasm, the dorsal part of the central grey (midbrain) and several components of the hippocampal formation, such as the subiculum, the CA3 field of the hippocampus proper, and the fornix. The structure that showed the greatest number of foci within both sides of the brain compared to the controls was the dorsal part of the central grey of the midbrain.

DISCUSSION

The results of this study clearly showed that following inoculation with fresh spinal cord in complete Freund's adjuvant, specific structures within the Lewis rat brain displayed higher densities of foci of lymphocytes than other regions. For the 15 structures with the largest numbers of foci per unit area, five were located within the hypothalamus, six were located within the midline thalamus,

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Table 4. Structures whose discrepancies exceeded +/− 3 standard deviations between the observed numbers and predicted numbers of foci of infiltrations of lymphocytes for rats exposed nocturnally to amplitude-modulated 500 nT, 7 Hz magnetic fields relative to controls

Structure	Observed	Predicted	z-resid
Right Side (r = .84, S.E. = .32)			
Trapezoid body nucleus	1.50	.12	4.22
Optic chiasm	8.25	5.62	7.34
Paraventricular thal. Nuc.(ant)	1.13	.11	3.08
Ventromedial hypothalamus	1.50	.25	3.83
Triangular septal nucleus	1.75	.50	3.81
Paranigral nucleus	1.00	2.62	−4.96
Pontine reticular nucleus	0	1.97	−6.06
Central acqueductal grey	0	1.08	−3.31
Left Side (r = .80, S. E. = .36)			
Optic chiasm	8.25	5.62	7.34
Medial habenular nucleus	2.25	1.14	3.08
Dorsomedial hypothalamus	1.50	.32	3.28
Posterior hypothalamus area	1.75	.01	4.89
Triangular septal nucleus	1.75	.52	3.45
Paranigral nucleus	.38	2.65	−6.37
Paraventic. Nuc. Hypo. Med parv.)	0	1.21	−3.37

four involved components of the major tracts involved with memory (fornix, septofimbrial nucleus), vision (optic chiasm) system, and propioception (medial lemniscus).

If the density of foci generally reflect the proficiency of function of these structures, then rats that develop EAE in general (without the contribution from exposures to the experimental magnetic fields) should exhibit altered: (1) visual acuity (even as albino animals), (2) proprioceptive feedback for muscle and limb position (medial lemniscus), (3) memory (the fornix), (4) circadian periodicities (suprachiasmatic nucleus), and, (5) consumption of fluids secondary to general distress (the paraventricular hypothalamus). These behaviors can be argued to actually define the major consequences or the correlates of EAE in the Lewis rat.

The effects of exposures to the four different types of experimental magnetic fields were superimposed upon this intricate pattern of densities of foci determined by the process of EAE and their interactions with the vulnerable regions of brain space. The 15-day exposures to either 7-Hz or 40-Hz frequencies whose amplitudes were modulated between either 1 nT

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Table 5. Structures whose discrepancies exceeded +/− 3 standard deviations between the observed numbers and predicted numbers of foci of infiltrations of lymphocytes for rats exposed nocturnally to amplitude-modulated 50 nT, 7 Hz magnetic fields relative to controls

Structure	Observed	Predicted	z-resid
Right Side (r = .86, S.E. = .15)			
Central grey dorsal part	1.13	.29	5.49
Optic chiasm	3.93	2.86	6.96
Subiculum	.67	.01	4.33
CA3 of hippocampus	.60	.01	3.03
Globus pallidus	.47	.02	3.30
Pontine nucleus (oral part)	.33	1.00	−4.33
Fimbria	.27	.84	−3.71
Medial preoptic area	0	.52	−3.35
Left Side (r = 0.87, S.E. = 0.15)			
Central grey dorsal part	1.13	.31	5.68
Central nuc. Of inferior collic.	.47	.01	3.23
Fornix	2.33	1.81	3.52
Optic chiasm	3.93	3.05	5.99
Paranigral nucleus	.93	1.44	−3.47
Stria medullaris of thalamus	.33	.86	−3.58
Paraventric. Nuc. Thal. (anter)	.40	.99	−4.06
Medial preoptic area	.20	.65	−3.08

and 50 nT or 1 nT and 500 nT resulted in the accumulation of numbers of lymphocytic foci that deviated significantly from this "normal" pattern. Some brain regions were more affected than others.

That the groups of rats exposed to the different frequencies and intensities exhibited different clusters of structures within which the concentration of lymphocytic foci were significantly higher (or lower) suggests that the brain's response to complex-patterned magnetic fields may be more specific than previously suspected. If the distribution of the foci had been driven by either the intensity (50 nT or 500 nT) or the frequency (7-Hz or 40-Hz), then similarities would have dominated the differences between frequencies (regardless of intensity) or between intensity (regardless of frequency). This differentiation was not observed.

Rats that had been exposed to the 40-Hz, 500 nT showed more foci than expected within the central core of the midbrain while those that had been exposed to the 40-Hz, 50 nT fields showed the largest numbers of foci within the alveus around the hippocampus and the paratenial nucleus (of the thalamus) which has direct input into the hippocampus. On the other hand the group that

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had been exposed to the 7-Hz, 500 nT fields showed more infiltrations within hypothalamic regions including the optic chiasm while the group that had been exposed to the 7-Hz, 50 nT fields exhibited more foci within multiple hippocampal structures and the optic chiasm.

The likelihood that random or spurious variations contributed to this pattern is low. The residuals for the regression analyses for each side of the brain for each group that had been exposed to one of the four experimental treatments revealed marked intragroup consistency. For the group exposed to the 50 nT, 7-Hz fields the elevated density of foci occurred within the dorsal part of the central grey for both sides of the brain. For the group exposed to the 500 nT, 7-Hz field the elevated numbers of foci occurred with optic chiasm for both sides of the brain. A less robust bilateral symmetry was noted for the groups that had been exposed to the 500 nT and 50 nT, 40-Hz fields where more elevated numbers of foci were found within the central periaqueductal grey and alveus, respectively.

The overall strengths of the correlations between the numbers of foci within four groups of rats exposed to the different magnetic field patterns and the sham-field-exposed rats were similar for all conditions except those exposed to the 500 nT, 40-Hz fields. These correlations for both sides of the brain were about 0.60 and were significantly weaker than the approximately 0.80 magnitude coefficients noted for the other treatments. The diminished strength of the association strongly suggests that the effects of the 500 nT, 40-Hz fields may have produced sources of variance within the numbers of foci that were qualitatively different than the pattern measures in the other three conditions. However the smaller sample size from which the averages were calculated may have also been a factor.

The variables (structures) that displayed the largest residuals were not specific to the density of foci per se. If only the first four structures with the largest discrepancies between observed and predicted values for numbers of foci are considered for each condition than for the 40-Hz, 50 nT and 500 nT and the 7-Hz, 50 nT and 500 nT groups, respectively, there were (out of maximum of 8, for both sides) 0, 3, 4, and three structures from those with highest (top 10) density of foci. For these groups the numbers (of a maximum of 8) of structures from Table 1 that entered the equations were 3, 5, 7, and 7, respectively. Chi-squared analysis indicated that the groups exposed to the 7-Hz fields showed more of these structures within the equations than those exposed to the 40-Hz fields (chi-squared $= 5.23$, $p < .05$). The remainder of the structures were not included in the list because their densities were less than

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1 foci/mm^2. However none of the values for density of foci were excessively skewed for any of the structures.

There were two results that deserve consideration for possible clinical application. First, both sides of the brains for the groups exposed to the 7-Hz 50 nT or 500 nT fields showed greater numbers of lymphocytic foci within the optic chiasm. Considering the adjacency of this structure to the suprachiasmatic nucleus and its important role in the regulation of pineal-mediated circadian variations in melatonin levels (Warren & Cassone, 1995), the possibility of a direct effect of the 7-Hz, amplitude-modulated fields presented for about 6 min once per hour during the later scotophase should be considered. Interestingly we have found that rats in which epilepsy was induced by a single systemic injection of lithium and pilocarpine displayed more seizures when exposed nocturnally to these fields (Persinger, 1996). Melatonin exhibits antiepileptic properties (Costa-Lotufo et al., 2002; Mevissen & Ebert, 1998) and weak magnetic fields can suppress nocturnal melatonin levels (Lerchl, Honaka, & Reiter, 1991). An intricate relationship between very weak magnetic fields, melatonin, and the severity of symptoms of multiple sclerosis has been also suggested by Sandyk (1992).

Secondly, the increased density of lymphocytic foci within hippocampal-related structures for the group that had been exposed to the 7-Hz, 50 nT amplitude-modulated magnetic field would suggest alterations in the representation of spatial patterns associated with memory. If the assumption that areas in which the infiltration occurred reflect a direct effect of the specific, applied magnetic field is correct, then the mechanism by which these weak, amplitude-modulated, 7-Hz fields affect seizure activity and other functions might be re-evaluated. A specific, highly focal change in the blood brain barrier within the optic chiasm due to a unique cytoarchitecture or geometric organization of the crossing-fibers (chiasmatic), may have encouraged the infiltration of sensitized lymphocytes into this region. Alternatively, one cannot exclude the possibility that these fields may have affected a gene within the nuclei of oliglodendroglia that expressed a variant myelin-basic protein in a statistically more frequent manner to which the sensitized lymphocytes responded by selective chemotaxis.

Many of the other structures that displayed more foci than expected are also related to both light entrainment and the balance between body temperature and energy consumption. The anterior paraventricular thalamic nucleus helps modulate light-entrained circadian rhythms (Salazar-Juarez, Escobar, & Aguilar-Roberlo, 2002). The paraventricular thalamic nucleus is

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involved with stress-induced activation of limbic forebrain areas (Bubser & Deutch, 1999) and tunes the temperature-energy balance to the hypothalamic, limbic, and brain stem regions only in chronically stressed (such as evolving EAE) animals (Bhatnagar & Dallman, 1999). Its projections to the shell of the nucleus accumbens and prefrontal cortices (Pinto, Jankowski, & Sesack, 2003) and its likely role in cocaine-induced sensitization and locomotor activity (Young & Deutch, 1998) also suggest a powerful role of this structure in the development of contextual (environmental) addiction.

Perhaps the most consistent and strongest effect was the increase (by a factor of about four) of the density of the numbers of lymphocytic foci within the dorsal part of the central grey in the group of rats that had been exposed to the 7-Hz, 50 nT fields. This treatment was the one that also evoked the greatest suppression of the clinical signs of EAE and reduced the total numbers of foci within the whole brain. Considering the prominence of foci within the dorsal portion of the central grey (of the midbrain) and its adjacency to the inferior colliculus, one would hypothesize that the memory of spatial locations for auditory stimuli or the reflexes contributing to this function might be preferentially affected.

There are two observations that may be of theoretical interest. First, the groups exposed to the 500 nT amplitude-modulated, 40-Hz fields exhibited about six times the numbers of foci than expected within oral part of the pontine reticular nuclei, subceruleus nucleus, and the deep mesencephalic nucleus. The greater density of foci within the oral part of the pontine reticular nuclei was evident within the left side of the brain for groups exposed to the 40-Hz field modulated at either 500 nT or 50 nT.

This structure is involved with rapid eye movement (REM) and has been considered the nodal link for the complex network that harmoniously generates and maintains “dream sleep”. According to Reinoso-Suarez, de Andres, Rodrigo-Angulo, & Garzon, (2001), the Type I neurons found within this structure are activated by glutamate input from the contralateral nucleus, acetylcholine from the pedunculopontine tegmental nucleus, and GABA from the reticular thalamic nucleus on the ipsilateral side. Microinjections of carbachol (a cholinergic agonist) and an alpha-2 adrenergic inhibitor (Idazoxan) increased REM sleep while microinjections of muscimol (a GABAa agonist), L-NAME (a NOS inhibitor) and morphine decreased REM sleep. Clonidine (an alpha 2 adrenergic agonist) or norepinephrine suppressed REM sleep completely.

Llinas and Pare (1991) have shown that in the human brain the waking state and dream sleep are associated with coherent 40-Hz transcerebral fields

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that are phase-modulated in a rostral-to-caudal direction. If the applied 40-Hz, 500 nT amplitude-modulated fields employed in this study directly affected the neuronal source of this approximately 40-Hz process than one might expect changes in the local capillary density or permeability within the neurons and glial cells comprising the pontine structures. This change could increase the likelihood of infiltration of sensitized lymphocytes. One cannot discern at this time if this infiltration was opportunistic because of the vascular changes associated with the greater metabolic demands associated with REM sleep or because the 40-Hz fields directly interacted through resonance with the electronic characteristics of the network.

An increase of foci density by a factor of about six, especially considering the relatively small volumes of these structures, might be expected to result in substantial modification of either the temporal structure or the duration of subsequent periods of REM. Although this might be considered a verification that 40-Hz phase-modulations associated with dreaming intimately involves the pontine region, there are other explanations. If 60-Hz, power frequency sine-waves were amplitude-modulated during the night due to accidental or normal alterations in line-loads in dense industrial areas, then these results suggest that the claims by "sensitive" people of disrupted sleep and memory difficulties (due to diminished periods of REM) may have an empirical bases.

The second result of this study that may have significant theoretical implication was the spatial location of the increased densities of foci in the brains of rats exposed to the various spatial-temporal configurations of magnetic fields. As indicated in Figure 1, these regions were primarily around the ventricles although not usually immediately adjacent to the ventricles. The heterogeneous but systematic spatial pattern of altered infiltration of lymphocytes following exposures to the various magnetic fields within a volume (the brain) that has been considered by many theorists to be a "homogeneous" space suggests the latter assumption may not be correct. One simple model for this effect throughout the volume of the rat brain might involve an interaction between the centroid of the mass and volume of the primarily dielectric brain and the distributions of the more conductive ventricular fluid.

If the complexity of the spatial mosaic of residuals of numbers of foci for rats that were exposed to different intensities and frequencies of magnetic fields is reliable, then the brain, itself, might serve as a "sensory organ" by which many magnetic fields might be detected. If the hundreds of spatial aggregates defined as nuclei were considered the "receptor fields" and the individual nucleus was considered the "receptor" there would be myriad degrees of freedom or potential combinations within this matrix by which differential stimulation of

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these "receptors" could respond to a continuum of extremely low frequency magnetic fields whose temporal and spatial structures could contain substantial and biologically-relevant information.

REFERENCES

Bhatnagar, S., & Dallman, M. F. (1999). The paraventricular nucleus of the thalamus alters rhythms in core temperature and energy balance in a state-dependent manner. *Brain Research*, *851*, 66–75.

Bubser, M., & Deutch, A. Y. (1999). Stress induced FOS expression in neurons of the thalamic paraventricular nucleus that innervate limbic forebrain sites. *Synapse*, *32*, 13–22.

Cook, L. L., & Persinger, M. A. (1999). Infiltration of lymphocytes in the limbic brain following stimulation of subclinical cellular immunity and low dosages of lithium and a cholinergic agent. *Toxicology Letters*, *109*, 77–85.

Cook, L. L., & Persinger, M. A. (2000). Suppression of experimental allergic encephalomyelitis is specific to frequency and intensity of nocturnally applied, intermittent magnetic fields in rats. *Neuroscience Letters*, *292*, 171–174.

Costa-Lotufo, L. V., Fonteles, M. M., Lima, I. S., de Oliveira, A. A., Nascimento, V. S., de Bruin, V. M. S., et al. (2002). Attenuating effects of melatonin on pilocarpine-induced seizures in rats. *Comparative Biochemistry and Physiology*, *131*, 521–529.

Lerchl, A., Honaka, K. O., & Reiter, R. J. (1991). Pineal gland "magnetosensitivity" to static magnetic fields is a consequence of induced electric currents (eddy currents). *Journal of Pineal Research*, *10*, 109–116.

Llinas, R. R., & Pare, D. (1991). On dreaming and wakefulness. *Neuroscience*, *44*, 521–535.

Mevissen, M., & Ebert, U. (1998). Anticonvulsant effects of melatonin in amygdala-kindled rats. *Neuroscience Letters*, *257*, 13–16.

Paxinos, G., & Watson, C. (1986). *The rat brain in stereotaxic coordinates*. Toronto: Academic Press.

Persinger, M. A. (1996). Enhancement of limbic seizures by nocturnal application of experimental magnetic fields that simulate the magnitude and morphology of increases in geomagnetic activity. *International Journal of Neuroscience*, *86*, 271–280.

Persinger, M. A., Cook, L. L., & Koren, S. A. (2000). Suppression of experimental allergic encephalomyelitis in rats by brief, hourly nocturnal pulses of nanoTesla complex magnetic fields that simulate geomagnetic activity. *International Journal of Neuroscience*, *100*, 107–116.

Pinto, A., Jankowski, M., & Sesack, S. R. (2003). Projections from the paraventricular nucleus of the thalamus to the rat prefrontal cortex and nucleus accumbens shell:

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Ultrastructural characteristics and spatial relationships with dopamine afferents. *The Journal of Comparative Neurology*, *459*, 142–155.

Reinoso-Suarez, F., de Andres, I., Rodrigo-Angulo, M. L., & Garzon, M. (2001). Brain structures and mechanisms involved with the generation of REM sleep. *Sleep Medicine Reviews*, *5*, 63–67.

Salazar-Juarez, A., Escobar, C., & Aguilar-Roberlo, R. (2002). Anterior paraventricular thalamus modulates light-induced phase shifts in circadian rhythmicity in rats. *American Journal of Physiological Regulation and Integrated Comparative Physiology*, *283*, 897–904.

Sandyk, R. (1992). Successful treatment of multiple sclerosis with magnetic fields. *International Journal of Neuroscience*, *66*, 237–250.

Warren, W. S., & Cassone, V. M. (1995). The pineal gland: Photoreception and coupling behavioral, metabolic, and cardiovascular circadian outputs. *Journal of Biological Rhythms*, *10*, 64–79.

Young, C. D., & Deutch, A. Y. (1998). The effects of thalamic paraventricular nucleus lesions on cocaine-induced locomotor activity and sensitization. *Pharmacology Biochemistry and Behavior*, *60*, 753–758.

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